STUDIES IN PYROPHILOUS FUNGI—II.*

CHANGES BROUGHT ABOUT BY THE HEATING OF SOILS AND THEIR RELATION TO THE GROWTH OF PYRONEMA AND OTHER FUNGI

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(WITH PLATES 24–26, CONTAINING 6 FIGURES)

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I. INTRODUCTION

Our observations noted in previous papers as to the occurrence of *Pyronema* on burned-over or heated soil had been previously noted by Kasaroff, who in this connection states that this striking phenomenon could not be explained on the assumption that the fungus had survived the heating, a subsequent infection being a much more likely explanation. The results of numerous experiments are not wholly consistent but point to certain conclusions.

Kasaroff thought that the absence of growth on unheated-soil is not due to the fact that through the process of heating more material necessary to the fungus is set free but rather that the unheated-soil contains constituents which render the growth of the fungus impossible, which constituents are destroyed by heat-

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ing. It was not found possible by washing with water to wholly remove from the unheated-soil the unfavorable constituent but the soil extract contained the substances of unheated-soil which are unfavorable to the growth of *Pyronema*. Experiments with heated-soil showed that the properties favorable to *Pyronema* growth which were developed in the soil by heating may be removed from the soil by washing and in other ways. The reason for this is not clear. By boiling the extract of an unheated-soil its unfavorable properties may be partially removed. An extract of heated-soil cannot, to any great extent, render an unheated-soil a favorable nutrient medium for *Pyronema* but this may be accomplished by the addition of kainite, while the addition of charcoal, coal, and coke in various forms yields no beneficial results.

II. TOXIN THEORY

The conclusions of Kasaroff as to the reasons for the failure of *Pyronema* to grow on unheated-soil while it thrives on heated-soil seem to indicate that the unheated-soil contains a substance toxic to *Pyronema* which substance is destroyed by heat. This explanation is strengthened by the fact that other investigators have found in soils substances toxic to the higher plants, which substances inhibit the growth of such plants even in the presence of an abundance of food material.

The idea that there are toxic organic materials in soils arising from previous plant growth or otherwise, is rather a new conception but one which has been advanced and confirmed by Schreiner and his collaborators in the last few years. According to these conceptions there are definite, organic compounds in the soil, which, in the case of four substances, have been isolated, crystallized and analyzed, thus proving their composition and constitution. Some of these substances were proved to be harmful to plants by water-culture experiments, and all of them belong to types of substances that may well prove toxic to different kinds of plants on further investigation.

Such organic bodies may arise in a variety of ways, by root excretions from the growing plants, by simple decomposition and oxidation of the plant remains in the soil, and by bacterial action, etc. When one remembers that the plant body contains besides

carbohydrates and proteins, the following more or less complex substances; alkaloids, glucosides, tannins, hydrocarbons, resins, etc., it is not difficult to imagine how compounds toxic to the plant might well arise either directly or through bacterial action, slow oxidation or other deep-seated changes. Schreiner has shown that the growth of a plant in a solution makes that solution rather toxic to the growth of the same plant in the same medium because of the throwing off of toxic organic substances, rather than the depletion of the food supply by the growth of the plant.

All of this shows that in questions of soil fertility for certain plants, we must not only consider what necessary food materials are present but also determine, if possible, whether there are any toxic substances present which would check plant growth even in the presence of an abundant supply of organic and inorganic food materials.

In order to test the toxin theory with reference to the growth of *Pyronema,* an extract of unheated-soil was prepared by adding four liters of distilled water to two kilograms of unheated-soil. This mixture was stirred frequently and allowed to stand for some time and later filtered and used for the treatment of heated-soil.

On November 11, 1909, a series of experiments was conducted in which three pots of soil heated to about 160° C. for about two hours (S^1, S^2, and S^3) were used, with a pot of similar soil unheated as a control (C). Pot (S^1) and control (C) were treated with distilled water. Pot (S^2) was treated with extract of unheated-soil prepared in the above manner, the mixture having been allowed to stand with frequent stirring for two hours. Pot (S^3) was also treated with extract of unheated-soil on the following day, the mixture having been allowed to stand for twenty-four hours instead of two as in the preceding experiment. Each was inoculated with *Pyronema.*

On November 23, (S^1) and (S^2) showed fair growth of *Pyronema* especially on the pots, but at this time no fruit had appeared, while (S^3) showed good growth of mycelium and fruit. Control (C) gave negative results as usual.

Other experiments similar to the above were later conducted. In each case the heated-soil treated with the extract of

unheated-soil prepared in various ways proved to be fully as favorable, as culture media for *Pyronema,* as similar soil treated with distilled water, the variations being no more marked than would be expected in experiments of this nature. Heated-soil treated with distilled water and that treated with the extract of unheated-soil both gave good results.

These results indicate that if the failure of *Pyronema* to grow on unheated-soil is due to toxic constituents present in the soil these substances are not soluble in water, at least not in sufficient quantities to render extracts of such soils toxic to *Pyronema.*

III. PYRONEMA GROWTH A FOOD PROBLEM

It had often been noted in the course of our experiments that heated-soil when watered had a peculiar and characteristic pungent odor, together with the rather pleasant odor of caramel. It had also been noted that heated-soil when watered, often assumed a darker color than the same kind of soil unheated and watered in the same manner. This change of color was not universal but frequently occurred and probably depended upon the intensity of the heat.

Having repeatedly failed to show that the extract of unheated-soil had any toxic influence on the growth of *Pyronema,* we were inclined to abandon the toxin theory as an explanation of the failure of this fungus to grow on unheated-soil. It then occurred to us to reverse our experiments and try the effect of heated-soil extract with unheated-soil (p. 115). On December 2, a five-inch pot of soil was heated to a temperature of 175° C. for about two hours. This soil was then cooled and the pot placed over a filter in an extraction apparatus arranged for this purpose. Distilled water was poured into the pot until it percolated through the soil and filtered into the bottom of the extraction apparatus. A similar pot of the same kind of soil unheated was treated in the same manner. The extracts of the heated-soil and unheated-soil made in this manner were very different; the extract of the heated-soil was of a bright amber or reddish-brown color and very clear, while that of the unheated-soil was clear or slightly clouded but with no trace of the color characteristic of the heated-soil extract. The odor of the heated-

soil extract was also very characteristic, approaching that of the heated-soil itself, while the unheated-soil extract had the odor of ordinary moist earth.

When concentrated by evaporation, the residue from the heated-soil extract was of a dark brown color and possessed a very strong odor of caramel.

Other extracts of local soils were made with similar results. When heated at low temperature the color of the soil extract is often very pale, while the same soils when reheated at a higher temperature yield a much more highly colored extract, which indicates that high temperatures are necessary factors in producing a highly colored extract.

The production of an extract from heated-soil of apparently different composition from that obtained from unheated-soils and which possessed the odor characteristic of soils most favorable to *Pyronema* growth, suggested the possibility that after all we were dealing with a *food* problem, notwithstanding the fact that this had apparently been disproved by Kasaroff.

In order to test the effect of heat on other than local soils a sample of North Dakota soil was obtained through the kindness of Professor H. L. Bolley of the North Dakota Agricultural College. A sample of this soil was heated and an extract made as in previous experiments. The results were similar in every way except that the extract was of a much darker color (reddish-brown), a result which would naturally be expected by reason of the large amount of organic material in the soil. A more detailed account of the results of these tests will be given in Section VII.

Extracts of a Massachusetts soil sent by the kindness of Mr. M. G. Clark did not differ materially from extracts of local New York soils.

We should call attention here to the correlation between the temperatures necessary for the production of a strong or highly colored extract and those necessary in order to render the soil favorable to the growth of *Pyronema*. It has already been noted in a previous paper that the higher the temperatures to which the soils are heated (so far as our experiments had gone), the more favorable are the conditions for the growth of the fungus. We

have since found that the higher the temperature to which the soil is heated (up to 200° C.) the more favorable are the conditions for the production of a highly colored extract. This would at least suggest that the formation of this extract is directly concerned in the growth of *Pyronema*.

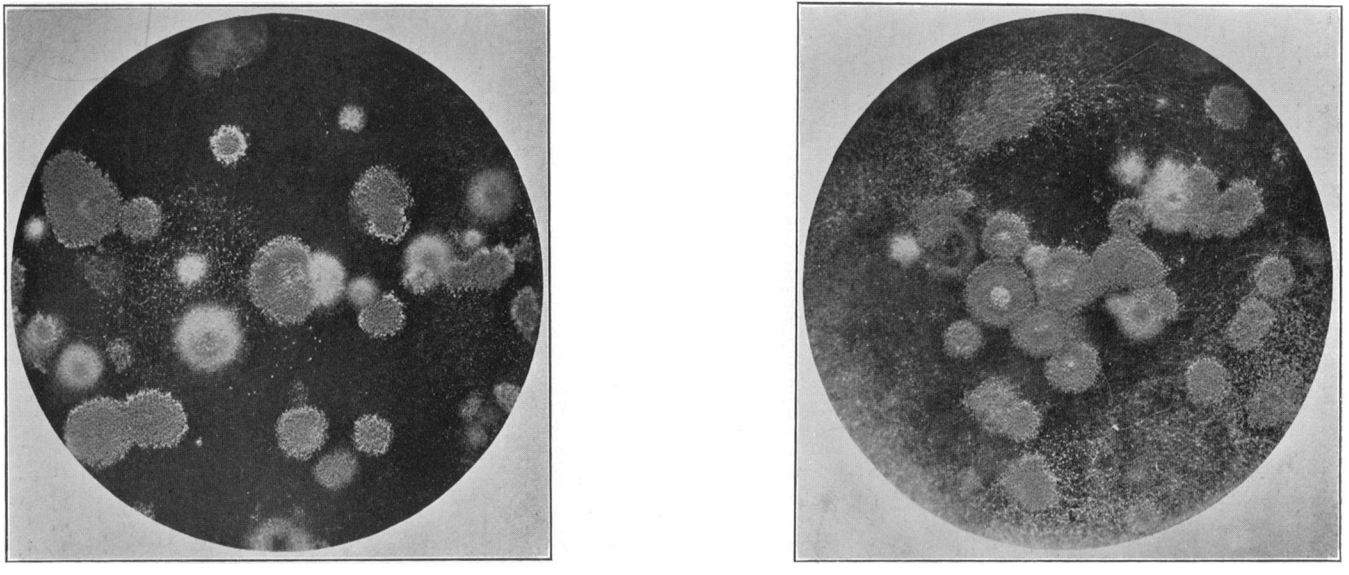
IV. BIOLOGICAL EXPERIMENTS WITH SOIL EXTRACTS

A number of extracts of heated soils were made and placed in tightly closed bottles, with no intention, however, of guarding against the possibility of air infection by fungi. Several of the extracts were evaporated down to various stages of concentration and all kept for experiment and study.

In a short time it was observed that a number of these extracts were infected with the mycelium of a fungus. In most cases the mycelium consisted of globose colonies varying from a few millimeters to a centimeter in diameter. Each consisted of a mass of mycelial threads radiating from a central point and apparently originating from a single spore. As the plants became older the mycelium became more fluffy and in some cases almost entirely filled the bottles containing the extract while in other cases the extract seemed to be less favorable for their continued growth. These colonies usually started near the bottom of the bottle, entirely immersed in the solution.

Some of this mycelium was removed from the bottles and placed on filter paper saturated with the extract. In several cases the fruit of *Pyronema* soon appeared and in some cases was produced in abundance, while in a few experiments no fruit was produced. From these experiments it became evident that the fungus which was infecting our extracts was *Pyronema,* as we had previously suspected and as the mycelium itself indicated.

So favorable are the heated-soil extracts as culture media for *Pyronema* that it has been found almost impossible to keep the extracts for any length of time in our laboratories without having them thoroughly infected with the fungus (*pl. 25, f. 2*). The same fact has already been noted with reference to heated-soil itself, this being so favorable as a nutrient medium for *Pyronema* that it is difficult to keep the fungus from invading



Two cultures of heated-soil extract self infected with fungi of various kinds, *Penicillium*, *Verticillium*, *Mucor* and *Pyronema*. Controls containing the extracts of the same soil before heating and exposed under the same conditions were entirely free from fungous infection.

these substrata when exposed in our laboratory where the spores are present from previous experiments.

As stated above, the extract of North Dakota soil is much darker in color than those of local soils, a result which would be expected since the soil itself is much blacker by reason of the large amount of organic matter present. We have shown by our experiments that the color of the extract is an index to the amount of soluble substances they contain. It would naturally follow that the extract of North Dakota soil is much richer and therefore a more favorable culture medium for *Pyronema* than the extract of the other soils studied, as our experiments have later shown.

In order to run a parallel test on the unheated-soil extract and heated-soil extract, three Petri dishes were partially filled with the highly colored extract of North Dakota soil and three similar dishes filled with the extract of the same soil unheated. These were placed side by side and loosely covered, allowing abundant opportunity for air infection. In a few days, the extract of the heated-soil showed an abundant infection consisting of numerous surface colonies of various kinds of fungi and a large number of immersed colonies of what appeared to be *Pyronema*. In about a week, two of the dishes showed abundant growth of *Pyronema,* the mycelium forming a very thin but tough membrane over the surface of the extract and the fruit being produced in great abundance over its surface, especially around the outsides of the culture (*pl. 24*). Although the mycelium of *Pyronema* had invaded our extracts continually these were the first cultures in which the fruit was produced in the extract. The controls containing the extract of unheated-soil showed no signs of infection by fungi of any kind.

The abundant infection of heated-soil extracts with *Pyronema* while the extracts of the same soil unheated remained uninfected, is strong evidence that the fungus appears here on account of the large amount of soluble food material liberated in the soil through the process of heating.

Numerous attempts to render unheated-soil favorable to *Pyronema* growth by the addition of heated-soil extract have failed to yield the expected results. Assuming that the extract contains food material for *Pyronema* it has been difficult to account for

these failures. There are however two possible explanations: (1) that the unheated-soil contains a toxin which retards the growth of the fungus even in the presence of abundant food material, which toxin is destroyed by heat, thus rendering our problem a double one; and (2) that the nature of the food material itself is changed by the action of the unheated-soil.

Our experiments indicate that these failures to render unheated-soil favorable for this fungus are due in part to the fact that the nature of the food material is changed when this is introduced into unheated-soil. This is shown by the fact that if a pot of unheated-soil is saturated with a concentrated extract of heated-soil (reddish-brown in color) and allowed to stand for a few days, it is found that the extract has almost entirely lost its color. This result may be due to chemical combination or to the adsorptive phenomena shown by many finely divided materials such as animal charcoal, which will completely decolorize large volumes of solutions containing dyes, etc. Quantitative studies of extracts treated in this manner show that the soluble materials have been reduced to approximately the same amount contained in extract of unheated-soil. This taking out of solution of the soluble materials in heated-soil extract when added to unheated-soil, seems to account for our failure to render unheated-soil favorable to *Pyronema* in this manner, but it is possible that there are other factors concerned. This is further suggested by the following observation: While it has been impossible to render unheated-soil favorable to *Pyronema* by the addition of heated-soil extract, heated-soil which is watered with the extract of other heated-soil is much more favorable than the same kind of soil treated with distilled water, as is shown by the fact that both mycelium and fruit of *Pyronema* are produced in much greater abundance on the heated-soil watered with the extract (*pl. 26*).

V. HEATED-SOIL AND ITS EXTRACTS AS NUTRIENT MEDIA FOR FUNGI

In a previous paper, attention was called to the fact that while heating of soil destroys fungi present at the time of heating, it prepares the way for the growth of those species which are intro-

duced subsequent to heating. This conclusion was drawn from the observation of such forms as *Verticillium, Fusarium,* and species of various other genera of the imperfect fungi which gain entrance to the soil through the planting of seeds. The growth of such fungi was much more abundant on heated- than on unheated-soil.

Our later experiments with heated-soil extracts confirm the above observation. In addition to *Pyronema* noted above, these extracts are immediately attacked by *Penicillium, Mucor, Aspergillus,* and a number of undetermined, imperfect fungi, which grow in abundance, entirely covering and filling the extracts, especially the stronger ones, while the extracts of the same soils unheated show no fungous growth whatever. The only way in which we have been able to preserve extracts of heated-soils in our laboratory is by sterilizing and tightly sealing them in bottles. In this way we have been able to preserve them in excellent condition, while if not sterilized and tightly sealed they are soon disintegrated through the action of bacteria and fungi (*pl. 25, f. 2*).

In our studies of the Iowa Discomycetes it has been observed that about five per cent. of the species of this group reported from Iowa occur only on burned places. That such habitats are unusually favorable to the growth of saprophytic fungi is beyond question.

It is likely that many of the beneficial results obtained through the sterilization of soils, which effects have been attributed to the destruction of harmful fungi and bacteria in the soil, are due more to the chemical changes accompanying sterilization than to the sterilization itself.

VI. DISTILLATES FROM HEATED-SOIL EXTRACTS

Some heated-soil extract of a brownish color was distilled to one half of its original volume and the distillate collected. The residual solution in the flask had the rather pleasant odor of the heated-soil extract but the distillate had the pungent odor which is also present in the original extract. Both the distillate and the liquid in the distilling flask were acid to litmus. We thought that possibly we had been able to separate the toxic from the non-toxic acid substances by their difference in volatility and for this reason

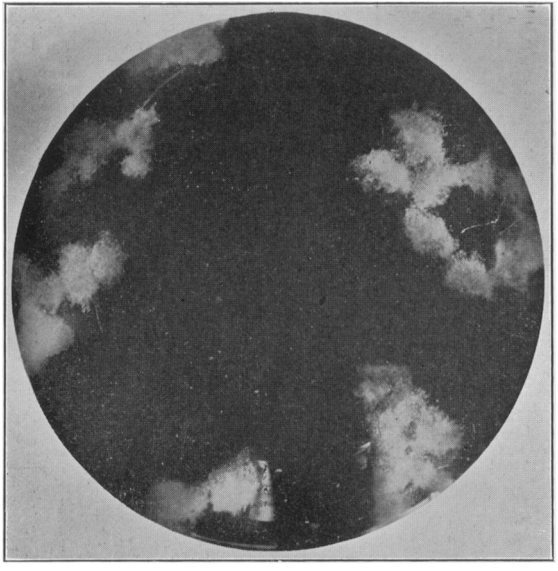
inoculated both liquids with the spores of *Pyronema.* In a week, the flask containing the dark-colored distillation residue was filled with the mycelium, while the other flask also had a considerable growth of *Pyronema,* the latter flask and liquid being perfectly transparent, with the silvery clumps of mycelium resting on the bottom of the flask (*pl. 25, f. 1*). Thus it seemed that distillation did not cause any appreciable separation of the substances in the extract, *i. e.,* judging from its effect on the growth of *Pyronema.*

VII. CHEMICAL STUDIES OF SOIL EXTRACTS

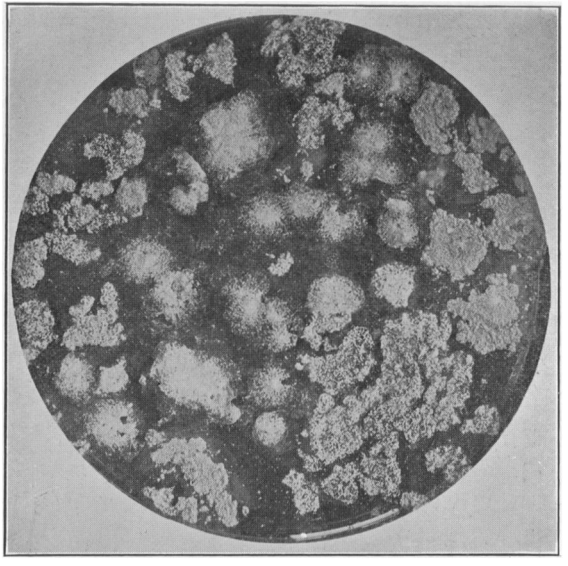
(*a*) QUANTITATIVE

As already noted, the color, odor, and general appearance of heated-soil extracts indicate that the composition of such extracts must be considerably different from that of extracts of unheated-soils. We decided to investigate first the quantitative differences between the extract of heated-soil and the same kind of soil unheated. The extracts which we analyzed were made by percolating the soils in 2 kg. samples with .2 liters of distilled water and taking 50 c.c. of the first liter of extract to come through, as the sample of the extract to be analyzed. The 50 c.c. samples of the extracts were evaporated to dryness in platinum dishes, dried at 108° to constant weight and this weight recorded as total solids. The residues were carefully ashed at a low, red heat, dried and weighed again, this weight recorded as inorganic matter and the difference between this weight and the weight of the total solids recorded as organic matter. We are aware that this method of determination of organic matter by difference of the weight obtained before and after ashing is not strictly accurate, but for the comparative purposes of this work this loss of weight may be used as a satisfactory measure of the organic matter present.

Determinations were made in this manner upon samples of New York soil, Massachusetts soil, and North Dakota soil. Since it had been noted that the percolation of an extract of heated-soil through an unheated-soil reduced and finally removed its color and again made it an unfavorable medium for *Pyronema* growth, we repeated this treatment with an extract of heated-soil whose composition was known and determined the change in com-



1. Colonies of *Pyronema* growing in the distillate of heated-soil extract.



2. Heated-soil extract infected with floating colonies of various kinds of fungi.

position after it had been acted on by the unheated-soil. The results of our work on the quantitative relations of soil extracts are presented in the following table:

INCREASE OF SOLUBLE MATTER IN SOIL UPON HEATING.

		Total solid matter. Per cent.	Organic matter. Per cent.	Inorganic matter. Per cent.
New York soil (1)	Unheated-soil extract	0.036	0.017	0.019
	Heated-soil extract	0.138	0.094	0.044
New York soil (2)	Unheated-soil extract	0.038	0.022	0.016
	Heated-soil extract	0.239	0.179	0.060
Massachusetts soil	Unheated-soil extract	0.016	0.008	0.008
	Heated-soil extract	0.249	0.197	0.052
North Dakota soil (1)	Unheated-soil extract	0.100	0.050	0.050
	Heated-soil extract	1.080	0.807	0.273
North Dakota soil (2)	Unheated-soil extract	0.101	0.037	0.064
	Heated-soil extract	0.986	0.758	0.228

DECREASE OF SOLUBLE MATTER IN HEATED-SOIL EXTRACT BY TREATMENT WITH UNHEATED-SOIL.

		Total solid matter. Per cent.	Organic matter. Per cent.	Inorganic matter. Per cent.
New York soil (2)	Before treatment	0.239	0.179	0.060
	After treatment	0.031	0.019	0.012
North Dakota soil (3)	Before treatment	0.756	0.576	0.180
	After treatment	0.100	0.052	0.048

In examining these results one is struck by the enormous increase of soluble matter produced by heating. This increase varies somewhat with different soils, depending upon the amount of organic matter present, the length of time heated, and the intensity of the heat, but in general the soluble matter in extracts of heated-soils is from six to ten times that contained in the extracts of the same soils before heating. The increase in the organic matter is greater than that in the inorganic matter, but still the latter is evidently increased several times. With such large amounts of both organic and inorganic matter made available in soils by heating, one can understand the preference of certain plants for places which have been burned over. It is interesting to note that where the heated-soil extract was percolated through and allowed to stand for a time with the unheated-

soil, the heated-soil extract was reduced to almost exactly the same condition as the extract of the same soil before heating.

(*b*) QUALITATIVE

We next undertook to investigate the nature of the substances that seemed to make heated-soil extracts favorable culture media for *Pyronema* and other fungi. The pungent odor of the extracts of heated-soil together with the pronounced acidity towards litmus suggested acids, while the dark color and caramel odor suggested carbohydrates or their decomposition products. The following tests were made on heated-soil extracts before being evaporated:

Litmus paper—red.

Lead acetate—brownish precipitate.

Silver nitrate—slight precipitate (not soluble in ammonia).

Barium chloride—slight precipitate.

Alcohol—slight precipitate.

Calcium hydroxide—slight precipitate.

Ether—does not dissolve color of solution.

Molisch test—positive.

This same extract when evaporated to one fiftieth its original bulk showed the same reactions in every case except that they were far more pronounced. This concentrated extract also caused a strong reduction of Fehling solution while the blanks were negative. All of the above tests were repeated many times and the results were practically always in accord with those described above.

When unheated-soil extracts were tested in exactly the same manner, the acidity was slight as shown by litmus, barium chloride gave a slight precipitate (owing to sulphates), and silver nitrate also gave a slight precipitate wholly soluble in ammonium hydroxide (probably owing to chlorides), while all the other tests were negative.

From the qualitative tests just described we are inclined to believe that upon heating to about 160° to 180° C., the organic matter in the soil undergoes some deep-seated changes probably oxidative in nature, favored by the high temperatures, which give us the water-soluble products of an acid character producing the dark-colored solutions. The acidity of heated-soil extracts

and the heavy precipitates obtained with lead acetate, silver nitrate, and calcium hydroxide, might well be due to the presence of organic acids. The positive Molisch test indicates carbohydrates or their decomposition products, while the strong reducing action on Fehling solution would seem to confirm the assumption that carbohydrate substances are present. It is not at all impossible that the partially disintegrated cellulose of the bodies of plants previously growing on the soil, would be broken up into still smaller fragments of the original enormous molecule, and that these smaller fragments would still retain some of their carbohydrate characteristics together with the added one of acidity.

We next examined the ash of the North Dakota heated-soil extract in a qualitative manner to discover if possible the nature of the inorganic substances in the extract. The ash of the North Dakota soil was used for the reason that this was obtained in considerable quantity and that the extract of this soil was unusually favorable as a culture medium for fungi, probably owing to the large amount of organic matter originally present in the soil. The ash of the *unheated* North Dakota soil was pure white and soluble in water (100 c.c.). Upon analysis the ash was found to consist principally of the sulphates of sodium, potassium, magnesium and calcium; we were however able to find scarcely a trace of phosphates. About one-half of the ash of the extract of *heated* North Dakota soil was found to be soluble in water. In the soluble portion of the ash, sulphates of potassium, sodium, magnesium and calcium, etc., were found. In the insoluble part of the ash, we found principally calcium sulphate with some manganese, iron and traces of phosphates, etc. Calcium is thus seen to be present in considerable quantities in the extracts of heated- and unheated-soils and it may be, from the well-known stimulating and protective properties of calcium toward plants, that this element along with the organic matter helps to give heated soils some of their striking properties.

Just as this work was being brought to a close we received, upon request, a copy of an article (in galley proof) by Professor T. L. Lyon, who is publishing the results of his investigations on the effects of steam sterilization upon soils. With steam heat he found the same great increase of soluble matter over

unheated-soils that we found in the case of dry heat at 180° C. He also calls attention to the same disappearance of this soluble organic matter when soils were allowed to stand after steam sterilization, that we had noted in our work on *Pyronema.* All this shows that either dry or steam heat may cause very important changes in soils and that it is to the effect of these changes on plants, as well as to the destruction of bacteria, etc., that we must ascribe the cultural results often noted in our experiments with heated-soils.

VIII. PRODUCTS OF DRY DISTILLATION OF SOIL

In order to see if the heating of a soil would drive off substances toxic to *Pyronema,* we filled a combustion tube with soil, put it in the furnace, fitted to the tube a smaller glass tube opening under a receiver of distilled water and heated the soil. Steam came over first and then more and more of a yellowish oil which was partially suspended in the water and partially formed a scum on the surface. The oily substance had an intensely irritating and nauseating odor like that of an old, stale pipe and recalled pyridine or its allies. The liquid in the receiver was alkaline to litmus. All of this seemed to indicate pyridine bases. We watered some heated-soil with this liquid and inoculated it with *Pyronema.* In a week, the growth on this soil was as good as that on the control watered with distilled water.

The soil left in the tube was black. This was watered with distilled water and inoculated with the spores of *Pyronema* but proved to be unfavorable to its growth, although some mycelium was produced.

IX. IDENTITY OF THE FUNGUS

The fungus which we have been cultivating in the laboratory had been determined by us as *Pyronema omphalodes* (Bull.) Fuckel, although its appearance in the laboratory differed slightly from specimens of this species previously observed by us in the field. In nature the ascocarps of this species give rise to dense, confluent masses in which it is difficult to recognize the individual ascocarps, while in the laboratory the plants are thickly gregarious but not confluent to the extent that they are in nature. It therefore occurred to us that the species might be distinct.

Both pots of soil heated, the one to the left watered with heated-soil extract and the one to the right with distilled water ; to show the improvement of heated soil for *Pyronema* by the addition of more extract.

In order to prove the identity of the species, in the spring of 1908 a pile of dead grass and leaves was raked together on the ground and burned, giving rise to a burnt place similar to those on which *Pyronema omphalodes* (Bull.) Fuckel was known to occur. As soon as the first rain occurred after the burning of this material a few of the plants from the laboratory were placed in the ashes and on the ground where the fire had been. In about ten days a good growth of *Pyronema omphalodes* (Bull.) Fuckel was found, the plants occurring in confluent masses as usual. These plants were taken into the laboratory and inoculations made from them on heated-soil. These at once produced mycelium radiating out from the point of infection and later produced an abundance of fruit, the ascocarps being scattered as is usually the case in laboratory grown material.

Some of the laboratory plants show rather well developed, hyaline, septate hairs, although these are not a conspicuous character. The color also varies much from bright rose or salmon to almost white. The paler plants are usually those produced on less favorable substrata. The fungus has doubtless been described under several names.

SUMMARY

1. Contrary to the statement of Kasaroff, our experiments have failed to show the presence of a soluble, toxic substance in unheated-soil which will retard the growth of *Pyronema* when applied to heated-soil.

2. Heating the soil to a high temperature brings about chemical changes indicated by the following: (*a*) The extract of heated-soil is of a bright amber or reddish-brown color and possesses a characteristic odor while the extract of unheated-soil is colorless and almost odorless, (*b*) the amount of soluble material in the extract of heated-soil is increased to approximately* six to ten times that of the extract of the same soil unheated.

3. The materials rendered available by the heating of the soil serve as food for *Pyronema,* as is indicated by the following: (*a*) The conditions necessary for the production of a highly colored extract in soil are the conditions most favorable to the growth

* The exact increase will vary with the soil and manner in which it is treated, temperature, length of time heated, etc.

of *Pyronema,* (*b*) the extract of heated-soil is itself so favorable as a culture medium that it is at once attacked by the fungus while the extract of the same soil unheated remains uninfected, (*c*) heated-soil watered with the extract of another heated-soil is much more favorable to *Pyronema* growth than similar soil watered with distilled water, the former producing mycelium and fruit in much greater abundance.

4. Distillation of heated-soil extract does not remove the properties favorable to *Pyronema,* both the colorless distillate and the highly colored distillation residue being favorable to its growth, the distillation residue, however, appearing to be more favorable than the distillate.

5. Excessive heating of soil in a combustion-tube renders it unfavorable to *Pyronema* growth. The distillate has a very offensive odor but is apparently neutral to *Pyronema* growth when applied to heated-soil.

6. It has been impossible to render unheated-soil favorable to the growth of the fungus by the introduction of the extract of heated-soil, this being apparently due to the fact that the nutrient materials in the extract are rendered insoluble by the action of unheated-soil.

7. Not only is the extract of heated-soil a favorable nutrient medium for *Pyronema,* but for other fungi as well, indicated by the fact that the extract is attacked by fungi of various kinds.

8. Soil subjected to steam or dry heat (either in a closed oven or by burning over the surface of the soil), becomes a very favorable nutrient medium for fungi of various kinds, by reason of the large quantity of food material rendered available through the heating of the materials in the soil.

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